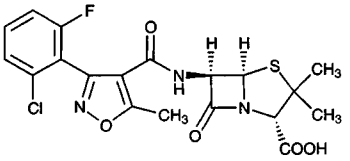
REFERENCE
Lee,S.C.; Renwick,A.G. Sulphoxide reduction by rat intestinal flora and by *Escherichia coli in vitro*, *Biochem.Pharmacol.*, **1995**, *49*, 1567–1576.

Floxacillin



Molecular formula: $C_{19}H_{17}ClFN_3O_5S$
Molecular weight: 453.88
CAS Registry No.: 5250-39-5, 1847-24-1 (sodium salt), 34214-51-2 (sodium monohydrate)
Merck Index: 4147

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 2 mL MeCN and 1 mL 10 mM pH 2 Na_2HPO_4. 250 µL Plasma + 100 µL 20 µg/mL dicloxacillin sodium in water, add 400 µL MeCN at -15° while vortexing, add 700 µL 10 mM pH 2 Na_2HPO_4, centrifuge at 8000 g for 10 min. Add the supernatant to the SPE cartridge, wash with 1 mL water, elute with two 500 µL portions of MeCN:water 35:65 containing 10 mM Na_2HPO_4 (pH adjusted to 6 with phosphoric acid), inject a 20 µL aliquot of the eluate.

HPLC VARIABLES
Column: 100 × 2 5 µm ODS Hypersil
Mobile phase: MeCN:water 40:60 containing 10 mM Na_2HPO_4, pH adjusted to 2 with ortho-phosphoric acid
Flow rate: 0.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 2.5
Internal standard: dicloxacillin (3.5)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: cloxacillin

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Hung,C.T.; Lim,J.K.C.; Zoest,A.R.; Lam,F.C. Optimization of high-performance liquid chromatographic analysis for isoxazolyl penicillins using factorial design, *J.Chromatogr.*, **1988**, *425*, 331–341.

SAMPLE
Matrix: blood
Sample preparation: 100 µL Plasma + 100 µL dicloxacillin in water + 25 µL glacial acetic acid + 2 mL ethyl acetate, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 70°, reconstitute the residue in 250 µL mobile phase, inject a 10-20 µL aliquot.

HPLC VARIABLES
Column: 40 × 3.2 RP18 VeloSep (Brownlee)
Mobile phase: MeCN:10 mM pH 7 phosphate buffer 18:82
Flow rate: 1.2
Injection volume: 10-20
Detector: UV 220

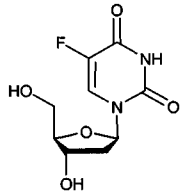
CHROMATOGRAM
Retention time: 2.8
Internal standard: dicloxacillin (4.4)
Limit of detection: 50 ng/mL
Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES
Simultaneous: carbamazepine, phenytoin, phenobarbital
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, caffeine, chloramphenicol, cyclosporine, digoxin, ethosuximide, gentamicin, lidocaine, nortriptyline, methotrexate, primidone, procainamide, quinidine, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin, metabolites

KEY WORDS
plasma

REFERENCE
Charles,B.G.; Foo,C.C.; Gath,J. Rapid column liquid chromatographic analysis of flucloxacillin in plasma on a microparticulate pre-column, *J.Chromatogr.B*, **1994**, *660*, 186–190.

Floxuridine



Molecular formula: $C_9H_{11}FN_2O_5$
Molecular weight: 246.20
CAS Registry No.: 50-91-9
Merck Index: 4148

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL water, vortex 10 s, add 200 μL water containing 30% w/v trichloroacetic acid and 30% w/v perchloric acid, vortex 10 s, place in an ice bath for 2 min, centrifuge at 3000 g for 20 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4.6 5μm Hypersil-ODS
Mobile phase: 20 mM Na_2HPO_4 adjusted to pH 2.0 with orthophosphoric acid
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: floxuridine

OTHER SUBSTANCES
Extracted: allopurinol, oxipurinol

KEY WORDS
plasma; floxuridine is IS

REFERENCE
Hung,C.T.; Zoest,A.R.; Perrier,D.G. Analysis of allopurinol and oxipurinol in plasma by reversed phase HPLC, *J.Liq.Chromatogr.*, **1986**, *9*, 2471–2483.

SAMPLE
Matrix: blood

Sample preparation: 100 μL Serum + 5 μL 42 μM 5-chlorouracil in acetone, mix, let stand at room temperature for 3 min, add 500 μL 100 mM pH 3.5 potassium phosphate buffer, add 2 mL ethyl acetate, vortex for 2 min, centrifuge at 1000 g for 5 min. Remove a 1.4 mL aliquot of the organic layer and evaporate it to dryness under reduced pressure. Add 20 mg solid potassium bicarbonate:anhydrous sodium sulfate 1:7 to the residue, add 50 μL 1.3 mM 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone in acetone, add 50 μL 1.5 mM 18-crown-6 in acetone, heat at 50° for 20 min, cool, inject a 10 μL aliquot. (Silanize all glassware. Synthesize 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Reflux 5 g 4,5-dinitroveratrole in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 60 mesh iron powder and 20 mL concentrated HCl in small portions over 1 h, reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.5 M NaOH, extract several times with 200 mL portions of benzene. Combine the organic layers and evaporate them to dryness, add 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as very slightly pink needles (mp 240°) (Anal. Chim. Acta 1982, 134, 39). Heat 2.5 mmoles 1,2-diamino-4,5-dimethoxybenzene hydrochloride and 2.4 mmoles pyruvic acid in 30 mL 500 mM HCl on a boiling water bath for 2 h, cool with ice-water, filter. Wash the precipitate with water and dry it under vacuum, recrystallize from MeOH:water 90:10 to give 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone as yellow needles (mp 255°) (Chem. Pharm. Bull. 1985, 33, 3493). Treat 1 g 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone dissolved in 50 mL anhydrous MeOH with a solution of diazomethane in ether, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using n-hexane:ethyl acetate 25:75 to give 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone as yellow needles (mp 170-171°). Dissolve 350 mg 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone in 3 mL acetic acid, add 350 mg anhydrous sodium acetate, add 2 mL 1.5 M bromine in acetic acid, heat at 100° for 15 min, cool, add 10 mL ether, filter, wash the solid 2 or 3 times with small portions of ether. Combine the filtrate and washings and evaporate them to dryness, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using ether, evaporate the main fraction to dryness, recrystallize the residue from n-hexane:ethyl acetate 50:50 to give 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as yellow needles (mp 161-163°) (J. Chromatogr. 1985, 346, 227). 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone is also available from Dojindo Molecular Technologies, Inc., 3 Bethesda Metro Center, Suite 700, Bethesda MD 20814; (301) 664-8448; www.dojindo.co.jp.)

HPLC VARIABLES

Column: 100 × 8 10 μm Radial Pak C18 (Waters) (Wash with MeOH at 2 mL/min for 20 min at the end of each day.)
Mobile phase: Gradient. MeOH:water 35:65 for 15 min, 50:50 for 25 min (step gradient), re-equilibrate at initial conditions for 20 min.
Flow rate: 1.5
Injection volume: 10
Detector: F ex 370 em 455

CHROMATOGRAM

Retention time: 28.1
Internal standard: 5-chlorouracil (32.5)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Extracted: 5-fluorouracil

KEY WORDS

derivatization; serum; pharmacokinetics

REFERENCE

Yamaguchi,M.; Nakamura,M.; Kuroda,N.; Ohkura,Y. Determination of 5-fluorouracil and 5-fluoro-2'-deoxyuridine in human serum by high-performance liquid chromatography with fluorescence detection, *Anal.Sci.*, **1987**, *3*, 75–79.

SAMPLE
Matrix: blood
Sample preparation: Add 250 μL serum to a 20 × 7 DEAE-Cellulofine AM anion-exchange column (Seikagaku Tokyo), elute with 3.5 mL 1 mM HCl, discard the first 0.5 mL eluate, collect the next 3 mL eluate. Evaporate the eluate to 0.5 mL under reduced pressure, add 15 mL ethyl acetate, shake, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 800 μL anhydrous acetone, add 100 μL 750 μg/mL 4-bromomethyl-6,7-dimethoxycoumarin in acetone, add 100 μL 250 μg/mL 18-crown-6 in acetone, add 1.5 mg anhydrous potassium carbonate, heat at 70° for 15 min (protect from atmospheric moisture with a calcium chloride drying tube), cool, inject an aliquot

HPLC VARIABLES
Column: 200 × 4 5 μm Nucleosil 5 C18
Mobile phase: MeOH:water 60:40
Flow rate: 0.8
Detector: F ex 340 em 420

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: ftorafur, 5-fluorouracil

KEY WORDS
derivatization; serum; protect from light; SPE

REFERENCE
Yoshida,S.; Adachi,T.; Hirose,S. 4-Bromomethyl-6,7-dimethoxycoumarin as a fluorescence reagent for precolumn derivatization of 5-fluorouracil compounds in high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *430*, 156–162.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL LC-SCX Supelclean strong cation-exchange SPE cartridge (Supelco) with 2 mL MeOH, 1 mL 100 mM copper(II) sulfate solution, and 3 mL 50 mM pH 7 phosphate buffer, do not allow to dry. 300 μL Serum + 5-bromouracil, add to the SPE cartridge, wash with 2 mL 50 mM pH 7 phosphate buffer, wash with 2 mL MeOH, elute with 700 μL 1.7 M ammonia solution, add 70 μL glacial acetic acid to the eluate, mix thoroughly, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-18-S (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-18-S ODS
Mobile phase: Gradient. A was MeOH:50 mM pH 6.5 phosphate buffer 60:40. B was 50 mM pH 6.5 phosphate buffer.
Flow rate: 1
Injection volume: 20
Detector: UV 269

CHROMATOGRAM
Retention time: 13
Internal standard: 5-bromouracil (12)
Limit of detection: 70 ng/mL

OTHER SUBSTANCES
Extracted: doxifluridine, 5-fluorouracil, 5-fluorouridine monophosphate, metabolites

KEY WORDS
serum; SPE

REFERENCE
Guerrieri,A.; Palmisano,F.; Zambonin,P.G.; De Lena,M.; Lorusso,V. Solid-phase extraction of fluoropyrimidine derivatives on a copper-modified strong cation exchanger: determination of doxifluridine, 5-fluorouracil and its main metabolites in serum by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1993**, *617*, 71–77.

SAMPLE
Matrix: blood, peritoneal fluid
Sample preparation: Plasma. 1 mL Plasma + 10 μL 100 μM bromouridine + 70 μL perchloric acid, mix thoroughly, let stand at 4° for at least 12 h, centrifuge for 5 min. Remove the supernatant and adjust the pH to 7 with 5 M KOH, let stand on ice for 2 h, inject a 20 μL aliquot. Peritoneal fluid. 1 mL Peritoneal fluid + 10 μL 100 μM bromouridine, dilute 1:100 with water, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 12.5 × 4.6 5 μm Zorbax RX
Column: 250 × 4.6 5 μm Zorbax RX
Mobile phase: Gradient. A was 25 mL pH 2.5 ammonium phosphate. B was MeCN:25 mM pH 7.5 ammonium phosphate 7:93. A:B 100:0 for 5 min, to 0:100 over 10 min, maintain at 0:100 for 10 min, return to initial conditions over 1 min, re-equilibrate for 20 min.
Column temperature: 20
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 16-17
Internal standard: bromouridine (18)
Limit of detection: 2.5 nM

OTHER SUBSTANCES
Extracted: 5-fluorouracil

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Smith-Rogers,J.A.; Tong,W.P.; Duafala,M.E.; Markman,M.; Bertino,J.R. High-performance liquid chromatographic method for the simultaneous measurement of floxuridine and fluorouracil in human body fluids, *J.Chromatogr.*, **1991**, *566*, 147–154.

SAMPLE
Matrix: blood, tissue
Sample preparation: Add 100 μL 10% perchloric acid and 20 μL 200 μg/mL IS to 100 μL serum or homogenized tissue. Shake for 2 min and centrifuge at 2000 g for 10 min. Filter (45 μm) supernatant, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 45 × 4.6 5 μm ODS Hypersil (VDS Optilab)
Column: 250 × 4.6 5 μm ODS Hypersil (VDS Optilab)
Mobile phase: MeOH:99% acetic acid:water 3:0.5:96.95
Column temperature: 30
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 12.75
Internal standard: 5-bromouracil (10.34)
Limit of quantitation: 200 ng/mL (serum); 600 ng/mL (tissue)

OTHER SUBSTANCES
Extracted: metabolites, fluorouracil

KEY WORDS
serum; rat; liver; tumor; kidney; spleen; peritoneum; gastric mucosa; lung; heart; pancreas

REFERENCE
Jung,M.; Berger,G.; Pohlen,U.; Päuser,S.; Reszka,R.; Buhr,H.J. Simultaneous determination of 5-fluorouracil and its active metabolites in serum and tissue by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *702*, 193–202.

SAMPLE
Matrix: blood, tissue
Sample preparation: 0.5 g Tissue or 1 mL plasma + 5 μL 1 M sulfuric acid (lung and heart only) + 2 μL 1 M sulfuric acid (plasma only) + 500 μL 200 mg/mL sodium sulfate (liver and kidney only) + 50 μL 1 M pH 6 sodium acetate (liver only) + 50 μL 1 M pH 5 sodium acetate (kidney only) + 100 μL 2% trichloroacetic acid (lung and heart only) + 15 mL n-propanol:ether (liver 16:84, kidney 20:80, lung 88:12, heart 40:60, plasma 88:12), sonicate for 30 s, shake for 15 min, centrifuge for 15 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 1 mL 50 mM ammonium phosphate (liver pH 11, kidney pH 3, lung pH 2.5, heart pH 5, plasma pH 2.5), inject a 20 μL aliquot. (From J. Liq.Chromatogr. 1994, 17, 1621.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb 5 ODS 2
Mobile phase: MeCN:50 mM phosphate buffer 0.5:99.5 (Liver pH 3, kidney pH 6, lung pH 5, heart pH 5, plasma pH 2.5)
Column temperature: 10 (kidney), 35 (lung), 20 (heart), 25 (plasma), 15 (liver)
Flow rate: 1
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 18 (plasma), 28 (lung), 30 (liver), 32 (kidney), 36 (heart)
Internal standard: flucytosine (for plasma) (4), 4-chlorouracil (for tissue) (17 (lung), 18 (liver), 11 (kidney), 19 (heart))
Limit of quantitation: 670 ng/g plasma, 110 ng/g (heart), 90 ng/g (lung), 210 ng/g (kidney), 500 ng/g (liver)

OTHER SUBSTANCES
Extracted: metabolites, 5-fluorouracil

KEY WORDS
plasma; rabbit; liver; kidney; lung; heart; pharmacokinetics

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Pampliega,A.; Marinero,P.; Pozuelo,M. Determination of the concentrations of 5-fluorouracil and its metabolites in rabbit plasma and tissues by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *656*, 397–405.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 30 mm long 3 μm C18 (Perkin-Elmer)
Mobile phase: MeOH:water 0.5:95.5 (sic)
Flow rate: 1
Injection volume: 10
Detector: UV 268

CHROMATOGRAM
Retention time: 2

KEY WORDS
stability-indicating; saline; injections

REFERENCE
Smith,J.A.; Morris,A.; Duafala,M.E.; Bertino,J.R.; Markman,M.; Kleinberg,M. Stability of floxuridine and leucovorin calcium admixtures for intraperitoneal administration, *Am.J.Hosp.Pharm.*, **1989**, *46*, 985–989.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation 1:100 with water, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 4 35-60 μm Perisorb RP18
Column: 250 × 4 10 μm LiChrosorb RP18
Mobile phase: MeOH:300 mM sodium acetate 2:98
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Simultaneous: fluorouracil

KEY WORDS
injections; water

REFERENCE
Sadjak,A.; Wintersteiger,R. Compatibility of morphine, baclofen, floxuridine and fluorouracil in an implantable medication pump, *Arzneimittelforschung*, **1995**, *45*, 93–98.

SAMPLE
Matrix: solutions
Sample preparation: Prepare 50 mg/mL or 1 mg/mL solutions in 0.9% sodium chloride injection, dilute 1:1000 or 1:100 with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: Bakerbond C18
Mobile phase: MeOH:water 0.5:99.5
Flow rate: 1
Detector: UV 268

CHROMATOGRAM
Retention time: 4.6

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
stability-indicating

REFERENCE
Stiles,M.L.; Allen,L.V.,Jr.; Prince,S.J. Stability of deferoxamine mesylate, floxuridine, fluorouracil, hydromorphone hydrochloride, lorazepam, and midazolam hydrochloride in polypropylene infusion-pump syringes, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1583–1588.

SAMPLE
Matrix: solutions
Sample preparation: Add 10 mg of 4-bromomethyl-7-methoxycoumarin and 10 mg potassium carbonate to 5 mL of a solution in DMSO, after 5 min at room temperature add 4-nitrobenzoic acid.

HPLC VARIABLES
Column: 200 × 4 5 μm Nucleosil 5 C18
Mobile phase: MeCN:MeOH:water 5:29:66
Flow rate: 0.6
Detector: F ex 346 em 395

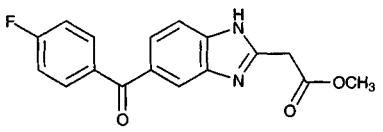
CHROMATOGRAM
Retention time: 12.5
Internal standard: chlorodeoxyuridine (16.5)

OTHER SUBSTANCES
Simultaneous: inosine, thymine, uracil, uridine

KEY WORDS
derivatization; some details of serum extraction in paper

REFERENCE
Yoshida,S.; Hirose,S.; Iwamoto,M. Use of 4-bromomethyl-7-methoxycoumarin for derivatization of pyrimidine compounds in serum analysed by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1986**, *383*, 61–68.

Flubendazole



Molecular formula: $C_{16}H_{12}FN_3O_3$
Molecular weight: 313.29
Merck Index: 4154
Lednicer No.: 2 354

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 16.745

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

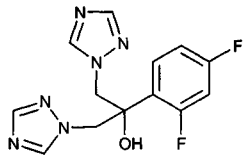
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole,

thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Fluconazole



Molecular formula: $C_{13}H_{12}F_2N_6O$
Molecular weight: 306.27
CAS Registry No.: 86386-73-4
Merck Index: 4158

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 700 μg/mL IS + 1.0 mL 1 M KOH, mix, extract twice with 3 mL portions of ethyl acetate. Combine the organic layers and extract with 2 mL 1 M HCl. Add 1.75 mL 1 M KOH to the aqueous layer and extract twice with 3 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of dry nitrogen, reconstitute the residue in 100 μL MeCN:10 mM pH 9.0 ammonium phosphate 50:50, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Microsorb C18
Mobile phase: Gradient. MeCN:10 mM pH 9.0 ammonium phosphate 10:90 for 1 min, to 60:40 over 15 min, maintain at 60:40 for 2 min.
Flow rate: 0.5
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 7
Internal standard: α-(2,4-dichlorophenyl)-1H-imidazole-1-ethanol (9)
Limit of quantitation: 12 μg/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Black,D.J.; Kunze,K.L.; Wienkers,L.C.; Gidal,B.E.; Seaton,T.L.; McDonnell,N.D.; Evans,J.S.; Bauwens,J.E.; Trager,W.F. Warfarin-fluconazole II. A metabolically based drug interaction: In vivo studies, *Drug Metab.Dispos.*, **1996**, *24*, 422–428.

SAMPLE

Matrix: blood
Sample preparation: Add 500 μL MeCN containing an excess of sodium carbonate to 500 μL plasma, vortex for 10 s, centrifuge at 2000 g for 10 min, dilute a 350 μL aliquot of the supernatant with 700 μL distilled water, inject an aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm Ultrabase C8 (SFCC, Neuilly Plaisance, France)
Mobile phase: MeCN:water 28:72
Flow rate: 1

Injection volume: 40
Detector: UV 260

CHROMATOGRAM
Retention time: 3.6
Limit of detection: 150 ng/mL
Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES
Noninterfering: amikacin, amoxicillin, carbamazepine, cilastatin, clavulanic acid, epoxycarbamazepine, flunitrazepam, imipenem, methotrexate, midazolam, ofloxacin, phenobarbital, phenytoin, piperacillin, teicoplanin, theophylline, thiopental, tobramycin, valproic acid, vancomycin

KEY WORDS
plasma

REFERENCE
Cociglio,M.; Brandissou,S.; Alric,R.; Bressolle,F. High-performance liquid chromatographic determination of fluconazole in plasma, *J.Chromatogr.B*, **1996**, *686*, 11–17.

SAMPLE
Matrix: blood
Sample preparation: Condition a 6 mL 500 mg Bakerbond C18 SPE cartridge with 2 column volumes of MeOH and 2 column volumes of buffer. 1 mL Plasma + 100 μL 100 μg/mL IS in buffer + 2 mL buffer, mix, add to the SPE cartridge, wash with 1 mL buffer, wash with 1 mL MeOH:buffer 15:85, dry under vacuum (8-9 inches Hg) for 1 min, elute with 500 μL MeOH without vacuum, elute with 500 μL MeOH under 3-4 inches Hg vacuum. Combine the eluates and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute with 200 μL mobile phase, inject a 15 μL aliquot. (Buffer was 100 mM pH 6.0 sodium phosphate buffer.)

HPLC VARIABLES
Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:25 mM pH 7.0 Tris buffer 25:75
Flow rate: 1
Injection volume: 15
Detector: UV 210

CHROMATOGRAM
Retention time: 5
Internal standard: 2-(2,4-difluorophenyl)-1-fluoro-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol (Pfizer UK-54373) (8)
Limit of detection: 100 ng/mL

KEY WORDS
plasma; comparison with capillary electrophoresis; pharmacokinetics; SPE

REFERENCE
von Heeren,F.; Tanner,R.; Theurillat,R.; Thormann,W. Determination of fluconazole in human plasma by micellar electrokinetic capillary chromatography with detection at 190 nm, *J.Chromatogr.A*, **1996**, *745*, 165–172.

SAMPLE
Matrix: blood, dialysate
Sample preparation: Plasma. Extract 100 μL plasma with 5 mL dichloromethane, centrifuge at 1000 g for 15 min. Evaporate the organic to dryness under a stream of nitrogen. Reconstitute the residue with 100 μL mobile phase, inject a 20 μL aliquot. Dialysate. Inject a 10 μL aliquot directly.

HPLC VARIABLES
Column: 200 × 2.1 5 μm HP ODS

Mobile phase: MeCN:buffer 13:87 (Buffer was 20 mm ammonium monobasic phosphate, adjusted to pH 7 with 5 M NaOH.)
Flow rate: 0.5
Injection volume: 10-20
Detector: UV 210

CHROMATOGRAM
Internal standard: UK-51,060 (plasma)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Yang,H.; Wang,Q.; Elmquist,W.F. Fluconazole distribution to the brain: a crossover study in freely-moving rats using in vivo microdialysis, *Pharm.Res.*, **1996**, *13*, 1570–1575.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

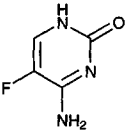
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 11.398

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Flucytosine



Molecular formula: $C_4H_4FN_3O$
Molecular weight: 129.09
CAS Registry No.: 2022-85-7
Merck Index: 4161

SAMPLE
Matrix: blood

Sample preparation: 500 μL Plasma + 50 μL 100 μg/mL 5-chlorouracil in MeOH + 30 μL 1 M pH 4.0 sodium acetate buffer + 300 μL 20% sodium sulfate in water + 8 mL diethyl ether:n-propanol 75:25, vortex for 5 min, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under vacuum using a freeze dryer, reconstitute the residue in 1 mL water, vortex, filter (0.22 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 5 μm YMC A-312 octadecylsilane (Yamamura Chemical)
Mobile phase: MeOH:50 mM pH 3.0 phosphate buffer 1:99
Flow rate: 1.5
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 8
Internal standard: 5-chlorouracil (3)
Limit of quantitation: 200 ng/mL

KEY WORDS
dog; plasma; pharmacokinetics

REFERENCE
Bonny,J.-D.; Kyowa,M. Use of in vitro release tests for the prediction of the in vivo behavior and the development of flucytosine controlled-release capsules, *J.Pharm.Sci.*, **1995**, *84*, 619–623.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.052

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations

Sample preparation: Dilute 30 μL of the injection to 100 mL with buffer, add 50 μL 10 mg/mL 5-flucytosine in water, inject a 20 μL aliquot. (Buffer contained 1 g/L $Na_2HPO_4.2H_2O$ and 0.363 g/L KH_2PO_4, pH 7.4.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC 18
Mobile phase: 10 mM KH_2PO_4 adjusted to pH 6.8 with 25% KOH
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.9
Internal standard: flucytosine

OTHER SUBSTANCES
Simultaneous: vidarabine phosphate

KEY WORDS
injections; water; flucytosine is IS

REFERENCE
Kwee,M.S.L.; Stolk,L.M.L. Formulation of a stable vidarabine phosphate injection, *Pharm.Weekbl.[Sci].*, **1984**, *6*, 101–104.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to 5 mg flucytosine, add 50 mL water, stir for 15 min, filter. 1 mL Filtrate + 1.5 mL 27 μg/mL thymine in buffer, make up to 10 mL with buffer, inject an aliquot. Injections. Dilute with water to a drug concentration of 100 μg/mL. 1 mL Sample + 1.5 mL 27 μg/mL thymine in buffer, make up to 10 mL with buffer, inject an aliquot. (Buffer was 70 mM KH_2PO_4 adjusted to pH 3.0 with HCl.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: 9 mM Sodium heptanesulfonate adjusted to pH 2.8 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 266

CHROMATOGRAM
Retention time: 6.3
Internal standard: thymine (4.85)

OTHER SUBSTANCES
Simultaneous: 5-fluorouracil

KEY WORDS
tablets; injections

REFERENCE
Cavrini,V.; Bonazzi,D.; Di Pietra,A.M. Analysis of flucytosine dosage forms by derivative UV spectroscopy and liquid chromatography, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 401–407.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 60 μL to 10 mL with 40 μg/mL p-aminobenzoic acid in MeCN: water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:buffer 25:75 (Buffer was 2 mL glacial acetic acid and 700 mg 1-octanesulfonic acid in 750 mL water.)
Flow rate: 1
Injection volume: 5
Detector: UV 285

CHROMATOGRAM
Retention time: 3.2
Internal standard: p-aminobenzoic acid (4.7)

OTHER SUBSTANCES
Noninterfering: degradation products

KEY WORDS
stability-indicating; oral liquids

REFERENCE
Wintermeyer,S.M.; Nahata,M.C. Stability of flucytosine in an extemporaneously compounded oral liquid, *Antimicrob.Agents Chemother.*, **1996**, *40*, 407–409.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 10 μm precolumn (Beckman Instruments Inc.)
Column: 150 × 4.6 5 μm C18 Altex Ultrasphere ODS
Mobile phase: Isocratic. MeOH:buffer 15:85 containing 2.5 mM sodium pentanesulfonate and 2.5 mM sodium heptanesulfonate. Gradient. A was MeOH containing 2.5 mM sodium pentanesulfonate and 2.5 mM sodium heptanesulfonate. B was buffer containing 2.5 mM sodium pentanesulfonate and 2.5 mM sodium heptanesulfonate. A:B 0:100 for 2 min, to 30:70 over 1 min, maintain at 30:70. (Buffer was 50 mM phosphoric acid containing 50 mM KH_2PO_4, pH 2.5.)
Flow rate: 1
Injection volume: 20
Detector: UV 254; UV 285

CHROMATOGRAM
Retention time: 3.76 (isocratic), 9.20 (gradient)
Internal standard: 5-methylcytosine (5.66 (isocratic), 12.62 (gradient))
Limit of quantitation: 12.5 μM

OTHER SUBSTANCES
Simultaneous: barbituric acid, cytosine, fluorouracil, hydroxycytosine, uracil, urea

REFERENCE
Biondi,L.; Nairn,J.G. High performance liquid chromatographic assay for 5-fluorouracil and 5-fluorocytosine, *J.Liq.Chromatogr.*, **1985**, *8*, 1881–1892.

SAMPLE
Matrix: solutions
Sample preparation: Centrifuge and filter cell solutions (0.22 μm), inject an aliquot.

HPLC VARIABLES
Guard column: Guard-PAK C18 (Waters)
Column: 150 × 3.9 5 μm NOVA PAK C18
Mobile phase: MeOH:50 mM pH 6.0 KH_2PO_4 3:97
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 2.5

REFERENCE
Koga,H. High-performance liquid chromatography measurement of antimicrobial concentrations in polymorphonuclear leukocytes, *Antimicrob.Agents Chemother.*, **1987**, *31*, 1904–1908.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

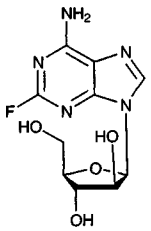
HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb 5 ODS 2
Mobile phase: MeCN:50 mM pH 2.5 phosphate buffer 0.5:99.5
Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: 5-fluorouracil, floxuridine

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Pampliega,A.; Marinero,P.; Pozuelo,M. Determination of the concentrations of 5-fluorouracil and its metabolites in rabbit plasma and tissues by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *656*, 397–405.

Fludarabine



Molecular formula: $C_{10}H_{12}FN_5O_4$
Molecular weight: 285.23
CAS Registry No.: 21679-14-1, 75607-67-9 (phosphate)
Merck Index: 4162
Lednicer No.: 4 167

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Bakerbond SPE cartridge packed with LiChrosorb RP-18 with 2 mL MeOH, 2 mL water and 1 mL MeOH. Mix 500 μL plasma with 100 μL 30 μg/mL mercaptopurine in MeOH, make up to 3 mL with MeOH. Centrifuge at 1100 g for 15 min, add 1.5 mL supernatant to the SPE cartridge, elute at 50 μL/min flow-rate, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 200 × 4 7 μm Lichrosorb RP-18
Mobile phase: MeOH: pH 4.15 phosphate buffer 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 6.30
Internal standard: mercaptopurine (3.30)
Limit of detection: 50 ng/mL
Limit of quantitation: 100 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Misztal,G.; Paw,B. Determination of fludarabine phosphate in human plasma using reversed phase high-performance liquid chromatography, *Pharmazie*, **1996**, *51*, 733–734.

SAMPLE
Matrix: blood
Sample preparation: Isolate mononuclear cells from 10 mL blood by a standard step-gradient density centrifugation procedure. Wash once with PBS, resuspend in 500 μL water, add 500 μL 800 mM perchloric acid, centrifuge at 400 g for 5 min, wash the pellet with 500 μL 400 mM perchloric acid, centrifuge at 400 g for 5 min. Combine supernatants, neutralize with 10 M KOH, bring to pH 7 with 1 M KOH (Universal indicator paper), cool in ice, centrifuge at 400 g for 5 min, inject a 50-2000 μL aliquot of the supernatant. (PBS was 8.1 g NaCl, 0.22 g KCl, 1.14 g $NaHPO_4$ (sic), 0.27 g KH_2PO_4 in 1 L water, pH 7.4.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil 10 SAX
Mobile phase: Gradient. A was 5 mM pH 2.8 $(NH_4)H_2PO_4$. B was 750 mM pH 3.5 $(NH_4)H_2PO_4$. A:B from 70:30 to 0:100 over 30 min (concave gradient, Waters no. 9). (At the start of each day pump through 20 mL 2 M $(NH_4)H_2PO_4$ then inject 100 μL 100 mM disodium EDTA into the initial mobile phase.)
Flow rate: 3
Injection volume: 50-2000
Detector: UV 262

CHROMATOGRAM
Retention time: 29 (as triphosphate, Fara-ATP)

OTHER SUBSTANCES
Extracted: ara-CTP (cytarabine triphosphate), ATP, CTP, UTP, GTP

KEY WORDS
mononuclear cells

REFERENCE
Gandhi,V.; Danhauser,L.; Plunkett,W. Separation of 1-β-D-arabinofuranosylcytosine 5'-triphosphate and 9-β-D-arabinofuranosyl-2-fluoroadenine 5'-triphosphate in human leukemia cells by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 293–299.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Filter (Amicon CF) while centrifuging at 1000 g for 20 min, filter (0.45 μm) the ultrafiltrate, inject an aliquot. Urine. 250 μL Urine + 500 μL 150 mM $Ba(OH)_2$, vortex, add 500 μL 5% $ZnSO_4.7H_2O$, vortex, centrifuge at 1700 g for 15 min, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:10 mM pH 4.15 $(NH_4)H_2PO_4$ 6:94
Flow rate: 1-1.2
Detector: UV 254

CHROMATOGRAM
Retention time: 55
Limit of detection: 12.5 pmole

OTHER SUBSTANCES
Extracted: fludarabine phosphate

KEY WORDS
plasma; pharmacokinetics; ultrafiltrate

REFERENCE
Hersh,M.R.; Kuhn,J.G.; Phillips,J.L.; Clark,G.; Ludden,T.M.; von Hoff,D.D. Pharmacokinetic study of fludarabine phosphate (NSC 312887), *Cancer Chemother.Pharmacol.*, **1986**, *17*, 277–280.

SAMPLE
Matrix: dialysate

HPLC VARIABLES
Column: 80 × 4.6 C18 (Perkin-Elmer)
Mobile phase: MeOH:10 mM pH 7 KH_2PO_4 20:80
Flow rate: 1
Detector: UV 265

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: araA, adenosine, 2-chloro-2'-arabinofluoro-2'-deoxyadenosine, 2-chloro-2'-deoxyadenosine, 2-chloroadenosine, 5'-chloro-5'-deoxyadenosine, 2'-deoxyadenosine

REFERENCE
Reichelova,V.; Liliemark,J.; Albertioni,F. Structure-activity relationships of 2-chloro-2'-*arabino*-fluoro-2'-deoxyadenosine and related analogues: Protein binding, lipophilicity, and retention in reversed-phase LC, *J.Liq.Chromatogr.*, **1995**, *18*, 1123–1135.

SAMPLE
Matrix: solutions

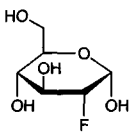
HPLC VARIABLES
Column: 80 × 4.6 3 μm (Perkin-Elmer)
Mobile phase: MeOH:10 mM pH 6.8 potassium phosphate buffer 20:80
Flow rate: 1
Detector: UV 265

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: cladribine, analogs, degradation products

REFERENCE
Reichelova,V.; Liliemark,J.; Albertioni,F. Liquid chromatographic study of acid stability of 2-chloro-2'-*arabino*-fluoro-2'-deoxyadenosine, 2-chloro-2'-deoxyadenosine and related analogues, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 711–714.

Fludeoxyglucose F18



Molecular formula: $C_6H_{11}{}^{18}FO_5$
Molecular weight: 181.15
CAS Registry No.: 105851-17-0
Merck Index: 4163

SAMPLE
Matrix: cell incubations

Sample preparation: Wash cells with PBS, freeze in liquid nitrogen, add 3 mL 400 mM perchloric acid, let stand at 4° for 30 min, centrifuge at 12000 g at 4°, extract with 3 mL 500 mM trioctylamine in 1,1,2-trichlorotrifluoroethane, filter (0.22 μm) the inorganic phase, inject an aliquot.

HPLC VARIABLES
Guard column: LiChrosorb RP 18-5
Column: 100 × 8 Radial-PAK Partisil 10 SAX (Waters)
Mobile phase: Gradient. A was MeOH:15 mM pH 3.8 $(NH_4)H_2PO_4$ 3:97. B was MeOH:750 mM pH 4.8 $(NH_4)H_2PO_4$ 3:97. A:B from 100:0 to 0:100 over 35 min (concave gradient), re-equilibrate for 15 min.
Flow rate: 2
Injection volume: 150
Detector: Radioactivity (Berthold LB 506 C-1) (with Quick Szint Flow 306 pumped at 4 mL/min)

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
C14 labeled; chondrocytes

REFERENCE
Fedders,G.; Kock,R.; Van de Leur,E.; Greiling,H. A radiochemical high-performance liquid chromatographic method for the analysis of 2-fluoro-2-deoxy-D-glucose-derived metabolites in human chondrocytes, *Anal.Biochem.*, **1993**, *211*, 81–86.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Evaporate reaction mixture in the presence of active charcoal and pass through a 100 × 10 column of Merck Kieselgel 60 for dry column chromatography using 70 mL MeCN:water 99.7:0.3, evaporate the eluate, take up the residue in 1 mL water, inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 3.6 Aminex HPX-87C (Bio-Rad)
Column: 300 × 7.8 Aminex HPX-87P (Bio-Rad) (The column contains lead which is washed off. Periodically backflush with 100 mM lead nitrate at 0.1 mL/min overnight.)
Mobile phase: Water
Column temperature: 20
Flow rate: 0.1
Detector: RI (at 18°)

CHROMATOGRAM
Retention time: 68.4

KEY WORDS
F18 labeled

REFERENCE
Oberdorfer,F.; Hull,W.E.; Traving,B.C.; Maier-Borst,W. Synthesis and purification of 2-deoxy-2-[18F]fluoro-D-glucose and 2-deoxy-2-[18F]fluoro-D-mannose: characterization of products by 1H- and 19F-NMR spectroscopy, *Int.J.Rad.Appl.Instrum.[A].*, **1986**, *37*, 695–701.

SAMPLE
Matrix: tissue hydrolyzate
Sample preparation: Centrifuge at 12000 g for 10 min, inject an aliquot.

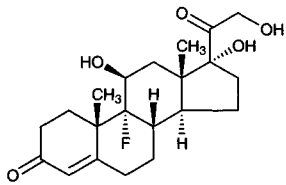
HPLC VARIABLES
Guard column: 20 × 4 APS Hypersil
Column: 250 × 4 BioSil Amino 5S (Bio-Rad)
Mobile phase: MeCN:water 90:10
Flow rate: 1
Detector: Radioactivity (with Quick Szint Flow 302)

CHROMATOGRAM
Retention time: 7

KEY WORDS
C14 labeled

REFERENCE
Fedders,G.; Kock,R.; Van de Leur,E.; Greiling,H. The metabolism of 2-fluoro-2-deoxy-D-glucose in human chondrocytes and its incorporation into keratan sulfate proteoglycans, *Eur.J.Biochem.*, **1994**, *219*, 1063–1071.

Fludrocortisone



Molecular formula: $C_{21}H_{29}FO_5$
Molecular weight: 380.46
CAS Registry No.: 127-31-1, 514-36-3 (acetate)
Merck Index: 4166
Lednicer No.: 1 192

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherex C18 (Phenomenex USA)
Mobile phase: MeOH:THF:water 3:25:72
Flow rate: 1.0
Injection volume: 60
Detector: UV 254

CHROMATOGRAM
Retention time: 15.9

OTHER SUBSTANCES
Simultaneous: 11-deoxycortisol, dexamethasone, hydrocortisone, methylprednisolone, prednisolone

REFERENCE
McWhinney,B.C.; Ward,G.; Hickman,P.E. Improved HPLC method for simultaneous analysis of cortisol, 11-deoxycortisol, prednisolone, methylprednisolone, and dexamethasone in serum and urine, *Clin.Chem.*, **1996**, *42*, 979–981.

SAMPLE
Matrix: solutions
Sample preparation: Inject 20 μL aliquot of a MeOH solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil 5-ODS
Mobile phase: THF:water 23:77
Column temperature: 30
Flow rate: 1

Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: k' 7.87 (fludrocortisone), k' 28.04 (fludrocortisone acetate)
Internal standard: methylprednisolone (k' 11.36)

OTHER SUBSTANCES
Simultaneous: metabolites, betamethasone, corticosterone, cortisone, deflazacort, deoxycorticosterone, dexamethasone, fluorocortisone, fluorocortisone acetate, hydrocortisone, 21-hydroxydeflazacort, 11α-hydroxyprogesterone, methylprednisolone, prednisolone, prednisone, triamcinolone acetonide, triamcinolone

REFERENCE
Santos-Montes,A.; Gonzalo-Lumbreras,R.; Gasco-Lopez,A.I.; Izquierdo-Hornillos,R. Extraction and high-performance liquid chromatographic separation of deflazacort and its metabolite 21-hydroxydeflazacort. Application to urine samples, *J.Chromatogr.B*, **1994**, *657*, 248–253.

SAMPLE
Matrix: urine
Sample preparation: Equilibrate a Sephadex G-25M column with 100 mM pH 7.0 phosphate buffer. Condition a Bond-Elut C18 SPE cartridge with 1 mL MeCN, 4 mL acetone:water 20:80, and 4 mL water. 2 mL Urine + 500 μL 500 mM pH 5.0 acetate buffer + 50 μL MeOH + 160 μL 100000 Fishmann U/mL β-glucuronidase and 800000 Roy U/mL arylsulfatase (from Helix pomatia, Boehringer Mannheim), heat at 37° for 24 h, filter (0.45 μm), add to the Sephadex column, wash with three 2 mL portions of 100 mM pH 7.0 phosphate buffer, elute with four 2 mL portions of 100 mM pH 7.0 phosphate buffer. Add the eluate to the SPE cartridge, wash with 4 mL water, wash with 4 mL acetone:water 20:80, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, add 20 μL cupric acetate solution, let stand at room temperature for 1 h, add 100 μL reagent, heat at 60° for 40 min, cool, centrifuge briefly at 1000 g, inject a 100 μL aliquot of the supernatant. (Cupric acetate solution was 0.7 g cupric acetate in water diluted to 100 mL with MeOH. Reagent was 7 mM 1,2-diamino-4,5-methylenedioxybenzene in water containing 200 mM β-mercaptoethanol and 250 mM sodium hydrosulfite, store in the dark at 4°, stable for at least 2 weeks. Prepare 1,2-diamino-4,5-methylenedioxybenzene as follows. Add 5 g 1,2-(methylenedioxy)-4-nitrobenzene to 37.5 mL concentrated nitric acid and 12.5 mL glacial acetic acid, pour the yellow-colored solution into water, recrystallize the 1,2-dinitro-4,5-methylenedioxybenzene from EtOH (Rec.Trav.Chim.Pays-Bas 1930, 49, 45). Dissolve 5 g 1,2-dinitro-4,5-methylenedioxybenzene in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 80 mesh iron powder, add 20 mL concentrated HCl in small portions over 1 h while heating the mixture under reflux. Reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.6 M NaOH, extract three times with 200 mL portions of benzene. Combine the extracts, evaporate to dryness to give 1,2-diamino-4,5-methylenedioxybenzene, mix with 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-methylenedioxybenzene dihydrochloride, mp 176-9° (Chem.Pharm.Bull. 1987, 35, 687).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm L-Column ODS (Chemicals Inspection and Testing Institute, Tokyo)
Mobile phase: MeOH:MeCN:500 mM ammonium acetate 50:10:40 (After each injection wash with MeOH:water 80:20 for 20 min for 20 min, re-equilibrate for 20 min.)
Flow rate: 1
Injection volume: 100
Detector: F ex 350 em 390

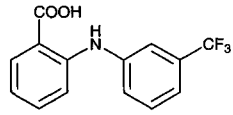
CHROMATOGRAM
Retention time: 35.6
Internal standard: fludrocortisone

OTHER SUBSTANCES
Extracted: hydrocortisone, tetrahydroaldosterone, aldosterone
Noninterfering: cortisone, corticosterone, hydroxycorticosteroids

KEY WORDS
SPE; derivatization; fludrocortisone is IS

REFERENCE
Yoshitake,T.; Ishida,J.; Sonezaki,S.; Yamaguchi,M. High performance liquid chromatographic determination of 3α, 5β-tetrahydroaldosterone and cortisol in human urine with fluorescence detection, *Biomed. Chromatogr.*, **1992**, *6*, 217–221.

Flufenamic acid



Molecular formula: $C_{14}H_{10}F_3NO_2$
Molecular weight: 281.23
CAS Registry No.: 530-78-9
Merck Index: 4167
Lednicer No.: 1 110

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 40 μL 100 μg/mL mefenamic acid in MeOH + 1 mL 1 M hydrochloric acid + 6 mL dichloromethane, shake mechanically for 20 min. Centrifuge at 2000 g for 5 min, evaporate organic phase to dryness under a gentle stream of nitrogen at 40°. Reconstitute the residue with 50 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 5 μm Nucleosil C18
Mobile phase: MeOH:water 77:23
Flow rate: 0.8
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 7.2
Internal standard: mefenamic acid (8.6)
Limit of detection: 100 ng/mL
Limit of quantitation: 500 ng/mL

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Cerretani,D.; Micheli,L.; Fiaschi,L.; Giorgi,G. High-performance liquid chromatography of flufenamic acid in rat plasma, *J.Chromatogr.B*, **1996**, *678*, 365–368.

SAMPLE
Matrix: formulations
Sample preparation: 100-300 mg Gel ointment + 3 mL MeOH, mix vigorously, filter (0.2 μm), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 4.3

KEY WORDS
ointment

REFERENCE
Yamamura,K.; Yamada,J.-I.; Yotsuyanagi,T. High-performance liquid chromatographic assay of antiinflammatory drugs incorporated in gel ointments. Separation and stability testing, *J.Chromatogr.*, **1985**, *331*, 383–388.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

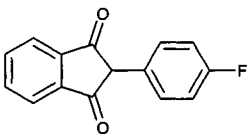
CHROMATOGRAM
Retention time: k' 1.35

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Fluindione



Molecular formula: $C_{15}H_9FO_2$
Molecular weight: 240.23
CAS Registry No.: 957-56-2
Merck Index: 4168

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL plasma to a siliconized tube containing 50 μL 2 μg/mL IS in MeOH and 100 μL MeCN. Vortex for 30 s, centrifuge at 3000 g for 10 min. Mix a 150 μL aliquot of the supernatant with 150 μL 0.9% NaCl, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:67 mM Na_2HPO_4 buffer adjusted to pH 7.2 with orthophosphoric acid 23:77 (At the end of each chromatographic session rinse the HPLC system with 200 mL MeCN:water 50:50.)
Flow rate: 1.5

Injection volume: 100
Detector: UV 280

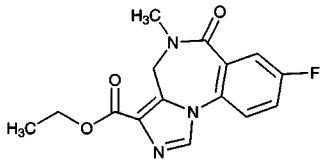
CHROMATOGRAM
Retention time: 4.2
Internal standard: coumarin (8.5)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Also analyzed: acenocoumarol, brodifacoum, bromadiolone, chlorphacinone, coumatetralyl, difenacoum, diphenadione, ethyl biscoumacetate, phenidione, warfarin

KEY WORDS
plasma

REFERENCE
Aymard,G.; Legrand,M.; Comets,E.; Mentre,F.; Diquet,B. Rapid and simple micromethod for the quantification of fluindione in human plasma using high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *707*, 169–173.

Flumazenil



Molecular formula: $C_{15}H_{14}FN_3O_3$
Molecular weight: 303.29
CAS Registry No.: 78755-81-4
Merck Index: 4169
Lednicer No.: 4 220

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 25 μL EtOH + 25 μL 3 μg/mL IS1 and 7.6 μg/mL IS2 in EtOH + 1 mL 100 mM Na_2HPO_4 adjusted to pH 10.5 with NaOH + 5 mL diethyl ether:dichloromethane 60:40, vortex for 30 s, centrifuge at 4° at 2000 g for 10 min. Remove the organic phase and add it to 1 mL 100 mM Na_2HPO_4 adjusted to pH 10.5 with NaOH, vortex for 30 s, centrifuge at 4° at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL mobile phase, inject a 1-15 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm CP-Microspher C18 (Chrompack)
Mobile phase: Gradient. A was MeOH:buffer 1:2. B was MeOH:water 80:20. A:b 93.8:6.2 for 5.5 min, to 60:40 over 0.15 min, maintain at 60:40 for 11.3 min, to 2.5:97.5 over 0.5 min, maintain at 2.5:97.5 for 3.5 min, return to initial conditions over 0.5 min (Buffer was 6 g/L NaH_2PO_4 and 1 mL/L triethylamine adjusted to pH 7.00 with NaOH.)
Column temperature: 40
Flow rate: 1.5
Injection volume: 1-15
Detector: UV 220

CHROMATOGRAM
Retention time: 5.0
Internal standard: IS1 ethyl 7-chloro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (Ro 15-305) (6.4), IS2 climazolam (17.0)
Limit of detection: 0.3 ng/mL

OTHER SUBSTANCES
Extracted: midazolam

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Vletter,A.A.; Burm,A.G.L.; Breimer,L.T.M.; Spierdijk,J. High-performance liquid chromatographic assay to determine midazolam and flumazenil simultaneously in human plasma, *J.Chromatogr.*, **1990**, *530*, 177–185.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum or plasma + 1 mL saturated Na_2HPO_4 + 100 µL 10 µg/mL IS + 5 mL diisopropyl ether:isopropanol 95:5 (Caution! Diisopropyl ether readily forms explosive peroxides!), shake for 5 min, centrifuge. Remove the organic layer and evaporate it to dryness at 35°, reconstitute the residue in 50 µL mobile phase, vortex, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 100 × 3 Chrompack CP spher C8
Mobile phase: MeCN:50 mM NaH_2PO_4 70:30 adjusted to pH 2.2 with 85% orthophosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 2.1
Internal standard: N-6-dimethyl-2-(4-methylphenyl)-N-propylimidazo[1,2-α]pyridine-3-acetamide methanesulfonate (Synthelabo France) (6.4)

OTHER SUBSTANCES
Extracted: prothipendyl, zolpidem

KEY WORDS
serum; plasma; pharmacokinetics

REFERENCE
Debailleul,G.; Khalil,F.A.; Lheureux,P. HPLC quantification of zolpidem and prothipendyl in a voluntary intoxication, *J.Anal.Toxicol.*, **1991**, *15*, 35–37.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with water, MeOH, and 100 mM ammonium acetate. Add 200 µL plasma to the SPE cartridge, wash with 100 mM ammonium acetate, elute with MeOH:100 mM ammonium acetate 3:1. Evaporate the eluate to dryness under reduced pressure, dissolve the residue in 200 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Hitachi gel 3056 octadecylsilica
Mobile phase: MeOH:100 mM ammonium acetate 60:40
Flow rate: 1
Injection volume: 20
Detector: MS, Hitachi M1000, APCI, nebulizer 260°, vaporizer 399

CHROMATOGRAM
Retention time: 2.7
Limit of detection: 0.5-2.5 ng/mL

OTHER SUBSTANCES
Simultaneous: atipamezole, atropine, butorphanol, ketamine, medetomidine, midazolam, xylazine

KEY WORDS
plasma; SPE; dog

REFERENCE

Kanazawa,H.; Nagata,Y.; Matsushima,Y.; Takai,N.; Uchiyama,H.; Nishimura,R.; Takeuchi,A. Liquid chromatography-mass spectrometry for the determination of medetomidine and other anaesthetics in plasma, *J.Chromatogr.*, **1993**, *631*, 215–220.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 244

CHROMATOGRAM

Retention time: 3.26

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or urine + 50 μL 4 μg/mL flurazepam in MeOH + 1 mL 100 mM pH 9 sodium phosphate buffer + 4 mL dichloromethane:diethyl ether 60:40, shake at 45 rpm for 15 min, centrifuge at 10° at 1870 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 80 μL MeOH, inject a 30 μL aliquot. (Deconjugate urine as follows. 250 μL Urine + 750 μL pH 5.4 acetate buffer + 500 U β-glucuronidase, heat at 37° for 18 h, add 20 μL 5 M NaOH, centrifuge, proceed as above using 5 mL dichloromethane:diethyl ether.)

HPLC VARIABLES
Guard column: 30 × 4.6 30 μm C8
Column: 100 × 8 4 μm Nova Pak C18
Mobile phase: MeCN:40 mM sodium phosphate buffer 32:68 containing 1 mL/L triethylamine, final pH 7.2
Flow rate: 1.5
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 3.8
Internal standard: flurazepam (18.5)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Extracted: midazolam, 4-hydroxymidazolam, 1-hydroxymethylmidazolam
Noninterfering: alfentanil, atropine, bupivacaine, lignocaine, neostigmine

KEY WORDS
plasma

REFERENCE
Chan,K.; Jones,R.D.M. Simultaneous determination of flumazenil, midazolam and metabolites in human biological fluids by liquid chromatography, *J.Chromatogr.*, **1993**, *619*, 154–160.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb C18
Mobile phase: MeOH:THF:isopropanol:water 30:3.5:1.5:65
Column temperature: 40
Flow rate: 1
Injection volume: 25
Detector: UV 245

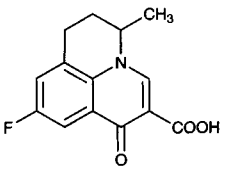
CHROMATOGRAM
Retention time: 6.30

OTHER SUBSTANCES
Simultaneous: methylparaben, propylparaben, phenol, degradation products
Noninterfering: aminophylline, cimetidine, dobutamine, dopamine, famotidine, lidocaine, procainamide, ranitidine

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Olsen,K.M.; Gurley,B.J.; Davis,G.A.; Christensen,R.; Monaghan,M.S. Stability of flumazenil with selected drugs in 5% dextrose injection, *Am.J.Hosp.Pharm.*, **1993**, *50*, 1907–1912.

Flumequine



Molecular formula: $C_{14}H_{12}FNO_3$
Molecular weight: 261.25
CAS Registry No.: 42835-25-6
Merck Index: 4172
Lednicer No.: 3 186

SAMPLE
Matrix: blood
Sample preparation: Mix 100 μL plasma with 100 μL pH 6.0 phosphate buffer and 1 mL ethyl acetate, shake, centrifuge at 20000 g for 5 min, collect 800 μL the organic phase, dry under nitrogen at 45°. Add 300 μL pH 7.8 phosphate buffer and 300 μL hexane to the residue, shake, centrifuge at 20000 g for 5 min, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 4 × 4 C18 (Merck)
Column: 125 × 4 5 μm Lichrospher RP Select B
Mobile phase: MeCN:DMF:water:orthophosphoric acid 18:28:40.5:13.5
Flow rate: 0.8
Injection volume: 100
Detector: UV 324 (A), F ex 320 em 365 (B)

CHROMATOGRAM
Retention time: 6.4
Limit of quantitation: 5 ng/mL (F), 100 ng/mL (UV)

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: ciprofloxacin, danofloxacin, enrofloxacin, marbofloxacin, nalidixic acid, oxolinic acid

KEY WORDS
sheep; plasma

REFERENCE
Delmas,J.M.; Chapel,A.M.; Sanders,P. Determination of flumequine and 7-hydroxyflumequine in plasma of sheep by high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *712*, 263–268.

SAMPLE
Matrix: tissue
Sample preparation: 2 g Minced tissue + 100 μL 5 μg/mL IS in water + 4 g anhydrous sodium sulfate, mix until homogenized. Add 10 ml ethyl acetate, shake mechanically for 10 min, centrifuge at 1500 g for 10 min. Transfer organic layer into another tube and repeat extraction on the tissue pellet with 10 mL ethyl acetate. Evaporate combined organic phases under a stream of nitrogen at 50°. Dissolve residue in 1 mL MeCN:2.7 mM pH 2.5 oxalic acid 50:50, vortex, sonicate for 5 min, filter through 0.45 μm filter (GHP Acrodisc GF, Gelman Sciences, USA). Inject a 100 μL aliquot. (1 mg/mL IS stock solution was prepared in 20 mM NaOH, it was diluted to working concentration with water immediately before use.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrabase C18 (Shandon, UK)

Mobile phase: Gradient. A was MeCN. B was 2.7 mM pH 2.5 oxalic acid. A:B from 10:90 to 70:30 over 20 min, maintain at 70:30 for 5 min, return to initial conditions over 5 min.
Flow rate: 0.8
Injection volume: 100
Detector: F ex 252 em 356

CHROMATOGRAM
Retention time: 20
Internal standard: ibafloxacin (21.3)
Limit of detection: 15 ng/g
Limit of quantitation: 50 ng/g

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
kidney; pig

REFERENCE
Guyonnet,J.; Pacaud,M.; Richard,M.; Doisi,A.; Spavone,F.; Hellings,P. Routine determination of flumequine in kidney tissue of pig using automated liquid chromatography, *J.Chromatogr.B*, **1996**, *679*, 177–184.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize 1 g tissue with 5 mL acetone, centrifuge, decant and save the supernatant, repeat the extraction. Add 2 mL acetone, 3 mL hexane, and 6 mL 3% NaCl to the combined supernatants, extract, centrifuge, discard the hexane layer. Add the extract to 25 mL chloroform (Caution! Chloroform is a carcinogen!), mix, separate the phases, add 2.5 mL 100 mM pH 9.0 Na_3PO_4 buffer and one drop 1 M NaOH to the chloroform phase, mix, separate the phases, discard the chloroform layer. Wash the aqueous layer with 2.5 mL chloroform, centrifuge the aqueous layer. Dialyze a 740 μL aliquot of the supernatant (in 2 portions) against 3.9 mL 20 mM pH 5.0 sodium phosphate buffer pumped at 0.6 mL/min using a Cuprophan 15000 MW cut-off cellulose acetate membrane. After washing column A with 500 μL MeCN:water 50:50 and 500 μL 20 mM pH 5.0 Na_3PO_4 buffer the dialysate flowed through column A to waste. Wash column A with 500 μL 20 mM pH 5.0 sodium phosphate buffer, elute the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B. (Wash the donor channel with 2 mL 0.01% Triton X-100 in 20 mM pH 5.0 sodium phosphate buffer. Wash the acceptor channel with 3 mL 20 mM pH 5.0 sodium phosphate buffer. Regenerate the membrane with 2 mL 100 mM pH 9.0 sodium phosphate buffer (donor channel) and 3 mL 20 mM pH 5.0 sodium phosphate buffer (acceptor channel).)

HPLC VARIABLES
Column: A 5.8 × 4.6 Hypersil ODS; B 150 × 4.6 PLRP-S (Polymer Labs., UK)
Mobile phase: MeCN:THF:20 mM pH 5.0 Na_3PO_4 buffer 20:15:65
Flow rate: 0.6
Detector: F ex 318 em 364

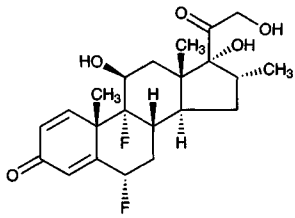
CHROMATOGRAM
Retention time: 12
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Extracted: oxolinic acid

KEY WORDS
column switching; chicken; liver; dialysis

REFERENCE
Eng,G.Y.; Maxwell,R.J.; Cohen,E.; Piotrowski,E.G.; Fiddler,W. Determination of flumequine and oxolinic acid in fortified chicken tissue using on-line dialysis and high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.A*, **1998**, *799*, 349–354.

Flumethasone



Molecular formula: $C_{22}H_{28}F_2O_5$
Molecular weight: 410.46
CAS Registry No.: 2135-17-3, 2002-29-1 (pivalate)
Merck Index: 4173
Lednicer No.: 1 200

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL serum to a Sep Pak C18 SPE cartridge, wash with 4 mL water, elute with 4 mL MeOH, evaporate to dryness under vacuum, reconstitute in 50 μL MeCN:water 30:70, inject whole sample.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water 30:70
Flow rate: 1
Injection volume: 50
Detector: enzyme immunoassay of fractions

CHROMATOGRAM

Retention time: 18
Limit of detection: 0.3 pg

OTHER SUBSTANCES

Extracted: dexamethasone, betamethasone, triamcinolone
Noninterfering: endogenous steroids

KEY WORDS

serum; SPE; horse

REFERENCE

Friedrich,A.; Schulz,R.; Meyer,H.H. Use of enzyme immunoassay and reverse-phase high-performance liquid chromatography to detect and confirm identity of dexamethasone in equine blood, *Am.J.Vet.Res.*, **1992**, *53*, 2213–2220.

SAMPLE

Matrix: blood
Sample preparation: Condition a Tef Elutor C18 cartridge with two 3 mL portions of MeOH then two 3 mL portions of water. Heat 1 mL plasma at 50° for 10 min, add to cartridge, wash with 2 mL water, 1 mL MeOH:water 10:90, 4 mL acetone:water 20:80, apply suction to cartridge for 10 min to air dry. Elute with 1 mL MeOH, evaporate eluent at 45° under nitrogen, reconstitute with 50 μL mobile phase, inject 25 μL aliquot.

HPLC VARIABLES

Column: 100 × 2 3 μm C18 Hypersil
Mobile phase: MeCN:THF:water 8:10:82, containing 5 mL/L triethylamine, pH adjusted to 6.5 with citric acid
Flow rate: 0.6
Injection volume: 25
Detector: UV 242

CHROMATOGRAM

Retention time: 11.50
Internal standard: flumethasone
Limit of detection: 300 pg/mL

OTHER SUBSTANCES

Simultaneous: dexamethasone, prednisone, hydrocortisone, adrenosterone, prednisolone, estriol, corticosterone, methylprednisolone, cortisone, hydroxyprogesterone, testosterone, deoxycorticosterone, fluorometholone, spironolactone, equilenin, estrone, estradiol, progesterone, diphenhydramine, propranolol, aspirin, theophylline, imipramine, desipramine, indomethacin, amitriptyline, nortriptyline, nordiazepam, diazepam, chlordiazepoxide, tripelennamine, carbamazepine, probenecid, phenobarbital

Noninterfering: caffeine, nicotine, cotinine, chlorothiazide, acetazolamide, phenytoin, pheniramine, cephalothin, primidone, acebutolol, hydrochlorothiazide, quinine, acetophenetidin, furosemide, aldosterone, triamcinolone, ephedrine, allopurinol, phenylephrine

KEY WORDS

plasma; SPE; flumethasone is IS

REFERENCE

Hariharan,M.; Naga,S.; VanNoord,T.; Kindt,E.K. Simultaneous assay of corticosterone and cortisol in plasma by reversed-phase liquid chromatography, *Clin.Chem.*, **1992**, *38*, 346–352.

SAMPLE

Matrix: blood

Sample preparation: Condition a 2 mL 200 mg Tef Elutor C18 SPE cartridge (Versa Prep) with 3 mL MeOH and two 3 mL portions of water. Heat 1 mL plasma at 50° for 10 min, add to SPE cartridge, wash with 2 mL water, wash with 1 mL MeOH:water 10:90, wash with 4 mL acetone:water 20:80, air-dry for 10 min, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 50 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 100 × 2 3 μm Hypersil

Mobile phase: MeCN:THF:water 8:10:82 containing 5 mL/L triethylamine, pH adjusted to 6.5 with citric acid

Flow rate: 0.6

Injection volume: 25

Detector: UV 242

CHROMATOGRAM

Retention time: 13

Internal standard: flumethasone

OTHER SUBSTANCES

Extracted: cortisone, corticosterone, hydrocortisone

Simultaneous: acebutolol, acetazolamide, acetophenetidin, adrenosterone, aldosterone, amitriptyline, androsten-3,17-dione, aspirin, carbamazepine, cephalothin, chlordiazepoxide, chlorothiazide, dehydrocorticosterone, deoxycorticosterone, deoxycortisol, desipramine, dexamethasone, diazepam, diphenhydramine, equilenin, estradiol, estriol, estrone, fluorometholone, furosemide, hydrochlorothiazide, hydroxycorticosterone, hydroxyprogesterone, hydroxyprogesterone, imipramine, indomethacin, methylhydroxyprogesterone, methylprednisolone, nandrolone, nordiazepam, nortriptyline, pheniramine, phenobarbital, phenytoin, prednisolone, prednisone, primidone, probenecid, progesterone, propranolol, quinine, spironolactone, testosterone, theophylline, triamcinolone, tripelennamine

Noninterfering: caffeine, nicotine, cotinine, ephedrine, allopurinol, phenylephrine

KEY WORDS

serum; SPE; flumethasone is IS

REFERENCE

Hariharan,M.; Naga,S.; VanNoord,T.; Kindt,E.K. Assay of human plasma cortisone by liquid chromatography: normal plasma concentrations (between 8 and 10 a.m.) of cortisone and corticosterone, *J.Chromatogr.*, **1993**, *613*, 195–201.

SAMPLE

Matrix: formulations

Sample preparation: Ointment. Weigh out 1-1.5 g ointment, add 10 mL chloroform:hexane 50:50, warm until the ointment dissolves, add to a silica Sep-Pak SPE cartridge, rinse container with 10 mL hexane:chloroform 50:50, add rinse to SPE cartridge, discard all eluates, rinse container with 5 mL MeOH, add rinse to SPE cartridge, elute with 15 mL MeOH, collect all MeOH eluates, make up to 25 mL with MeOH, inject a 25 μL aliquot. Cream. Weigh out 1 g cream and add it to 10 mL MeOH, shake for 5 min, sonicate for 5 min, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 C8 (Brownlee)
Mobile phase: MeOH:THF:water 30:30:40
Flow rate: 1.5
Injection volume: 10-25
Detector: UV 254

CHROMATOGRAM
Retention time: 8.4 (flumethasone pivalate)

KEY WORDS
ointment; cream; SPE

REFERENCE
Lodge,B.A.; Vincent,A. Analysis of flumethasone pivalate formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *301*, 477–480.

SAMPLE
Matrix: liposomal preparations
Sample preparation: Dilute 1000-fold with MeOH/water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: C18
Mobile phase: MeOH:1% acetic acid 70:30
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.12

OTHER SUBSTANCES
Interfering: dexamethasone

REFERENCE
Devoisselle,J.-M.; Vion-Dury,J.; Confort-Gouny,S.; Coustaut,D.; Cozzone,P.J. Liposomes containing fluorinated steroids: an analysis based on photon correlation and fluorine-19 nuclear magnetic resonance spectroscopy, *J.Pharm.Sci.*, **1992**, *81*, 249–254.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 4 mL water then 3 mL MeOH. Add aqueous steroid solution to cartridge, elute with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:water 70:30
Flow rate: 1
Injection volume: 20
Detector: UV 237

CHROMATOGRAM
Limit of detection: 120 ng/mL

OTHER SUBSTANCES
Also analyzed: betamethasone 17-valerate, dexamethasone

KEY WORDS
for flumethasone 21-acetate; SPE

REFERENCE
Valenta,C.; Janout,H. Corticosteroid analysis by HPLC with increased sensitivity by use of precolumn concentration, *J.Liq.Chromatogr.*, **1994**, *17*, 1141–1146.

SAMPLE
Matrix: synovial fluid
Sample preparation: 100 µL Synovial fluid + 10 µL 10 µg/mL flumethasone in MeOH + 1 mL 100 mM NaOH + 10 mL dichloromethane, shake for 10 min, centrifuge at 8400 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 µL mobile phase, vortex, inject the whole amount.

HPLC VARIABLES
Column: 100 × 8 10 µm Radial Pak B silica (Waters)
Mobile phase: Dichloromethane:MeOH:glacial acetic acid 96.8:2.4:0.8
Flow rate: 1.4
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: flumethasone

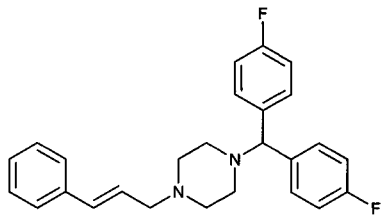
OTHER SUBSTANCES
Extracted: methylprednisolone, methylprednisolone acetate

KEY WORDS
normal phase; cow; flumethasone is IS

REFERENCE
Alvinerie,M.; Toutain,P.L. Determination of methylprednisolone and methylprednisolone acetate in synovial fluid using high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *309*, 385–390.

Flunarizine

Molecular formula: $C_{26}H_{26}F_2N_2$
Molecular weight: 404.50
CAS Registry No.: 52468-60-7, 30484-77-6 (2.HCl)
Merck Index: 4179
Lednicer No.: 2 31



SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

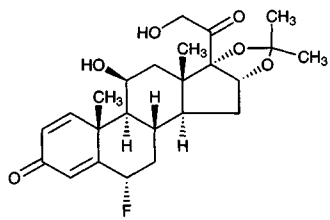
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 19.317

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Flunisolide



Molecular formula: $C_{24}H_{31}FO_6$
Molecular weight: 434.50
CAS Registry No.: 3385-03-3, 77326-96-6 (hemihydrate), 4533-89-5 (acetate)
Merck Index: 4180
Lednicer No.: 2 181

SAMPLE
Matrix: blood
Sample preparation: Add 30 μL IS to 1 mL serum, add 5 mL diethyl ether/dichloromethane (ratio not given), mix for 30 min. Centrifuge and freeze at -80° for 15 min. Evaporate the supernatant under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: Lichrospher RP Select B
Mobile phase: MeCN:20 mM ammonium acetate buffer 80:20
Flow rate: 1
Injection volume: 50
Detector: MS, PE-Sciex API 300, negative ion mode

CHROMATOGRAM
Internal standard: budesonide
Limit of detection: 100 pg/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Möllmann,H.; Derendorf,H.; Barth,J.; Meibohm,B.; Wagner,M.; Krieg,M.; Weisser,H.; Knöller,J.; Möllmann,A.; Hochhaus,G. Pharmacokinetic/pharmacodynamic evaluation of systemic effects of flunisolide after inhalation, *J.Clin.Pharmacol.*, **1997**, *37*, 893–903.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 10 μm Spherisorb ODS
Mobile phase: MeCN:water:acetic acid 35:64:1
Injection volume: 100
Detector: UV 240

CHROMATOGRAM
Internal standard: fluocinonide

KEY WORDS
nasal spray

REFERENCE
Yu,C.D.; Jones,R.E.; Henesian,M. Cascade impactor method for the droplet size characterization of a metered-dose nasal spray, *J.Pharm.Sci.*, **1984**, *73*, 344–348.

SAMPLE
Matrix: solutions

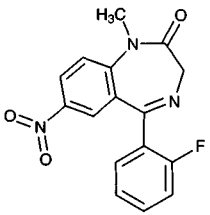
HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water 55:45
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 10.2

OTHER SUBSTANCES
Simultaneous: metabolites

REFERENCE
Tökés,L.; Cho,D.; Maddox,M.L.; Chaplin,M.D.; Chu,N.I. Isolation and identification of an oxidatively defluorinated metabolite of flunisolide in man, *Drug Metab.Dispos.*, **1981**, *9*, 485–486.

Flunitrazepam



Molecular formula: $C_{16}H_{12}FN_3O_3$
Molecular weight: 313.29
CAS Registry No.: 1622-62-4
Merck Index: 4181
Lednicer No.: 2 406

SAMPLE
Matrix: blood
Sample preparation: Vortex 1 mL plasma with 3 mL toluene:isoamyl alcohol 95:5 at 1000 rpm for 90 s, centrifuge at 2600 g for 10 min. Evaporate a 2.5 mL aliquot of the upper organic layer to dryness under nitrogen at 40°, reconstitute with 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Nucleosil 120-5 C18